STUDIES ON MEMBRANE TRANSPORT. II. THE ABSORPTION OF ACETATE AND BUTYRATE BY HYMENOLEPIS DIMINUTA (CESTODA) [1]

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Lipid metabolism in tapeworms has been studied recently by several investigators (Fairbairn *et al.*, 1961; Harrington, 1965; Ginger and Fairbairn, 1966a, b; Jacobsen and Fairbairn, 1967; Lumsden and Harrington, 1966; McMahon, 1961), and there is abundant evidence that *Hymenolepis diminuta* absorbs fatty acids from the external medium and incorporates them into lipid components of the tissues. Von Brand's hypothesis (1966), that the fatty acids in tapeworm tissues are waste products of carbohydrate metabolism, has not been supported by careful *in vivo* and *in vitro* experiments (Ginger and Fairbairn, 1966b; Jacobsen and Fairbairn, 1967), and all direct available evidence suggests that the lipids of *H. diminuta* are synthesized from fatty acids absorbed from the environment. On the other hand, although there is considerable evidence for the mediated absorption of amino acids, sugars, purines, and pyrimidines by this worm (reviewed by Read, 1966), there seem to be no published studies dealing with mechanisms for absorption of fatty acids by *H. diminuta* or other tapeworms. The present investigation was undertaken to characterize systems by which these presumably important nutrients may enter the tissues of some parasitic organisms.

Materials and Methods

Read, Rothman and Simmons (1963) described techniques for the maintenance and experimental manipulation of the tapeworm, *Hymenolepis diminuta*. With few exceptions these methods were adopted during the present investigation.

Young male rats (Holtzman Rat Co., Madison, Wisconsin), weighing 80–100 g. at the time of infection, were used as hosts in all experiments; animals received a diet of Purina Laboratory Chow. Ten days following infection with 30 cysticercoids (obtained from previously infected *Tenebrio molitor*), the rats were killed and the intestines removed. Parasites were flushed from the intestine with Krebs-Ringer solution containing 25 m*M* tris(hydroxymethyl)aminomethane-maleic acid buffer at pH 7.4. This solution (KRT) was used for all subsequent washes and, except as otherwise stated, as a solute in experimental incubations. After removal from the intestine, worms were washed and randomized in groups of five. They were then preincubated in 10 ml. KRT for a period of 30 minutes in a shaker bath at 37° C., after which each five-worm sample was transferred to 4 ml. of incubation solution for a period of one minute. Following incubation worms were washed in KRT, blotted on hard filter paper and dropped into 5 ml. 70% ethanol. After

[1] This work was supported by grants from the National Institutes of Health, U. S. Public Health Service, AI-01384 and 5 TI AI 106.

18–24 hrs. the worms were removed from the ethanol, dried overnight in an oven at 90° C. and weighed. Aliquots of the ethanol extract were mixed with 0.5 ml. of a 5 m*M* sodium bicarbonate solution prior to drying on aluminum planchettes and counting on a gas flow counter. Uptake data are based on the amount of radioactive material extracted in 70% ethanol and are expressed as μmoles/g./min., using the ethanol-extracted dry weight.

Sodium acetate and sodium butyrate labelled with ^{14}C at the C-1 position were obtained from New England Nuclear Co. and Nuclear Chicago Co., respectively. Unlabelled sodium salts of valeric acid (Eastman Organic Chemicals) and octanoic acid (Matheson, Coleman, and Bell) were prepared by titration of the appropriate acid to pH 7.6 with sodium hydroxide. Formate, acetate, propionate and butyrate were purchased as sodium salts of reagent grade.

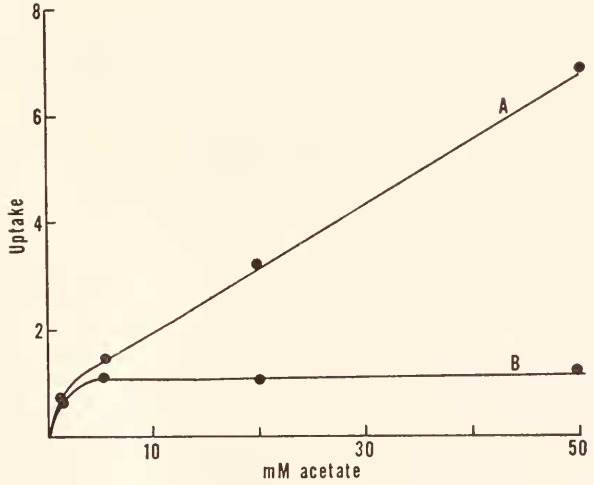

FIGURE 1. The uptake of ^{14}C-acetate as a function of acetate concentration. Curve A is uncorrected uptake. Curve B is uptake corrected by subtracting diffusion, calculated from the linear portion of Curve A. Each point is a mean of 4 determinations.

RESULTS

The relationship between acetate uptake and concentration is shown in Figure 1. The data indicate that the uptake system is not fully saturated even at the highest concentrations tested. At concentrations from 5 to 50 m*M* the uptake rate increases linearly with increase in concentrations, whereas below approximately 2 m*M*, acetate uptake is not a linear function of concentration. This suggests that a mediated transport mechanism operates during the uptake of acetate but, at high acetate concentrations, mediated transport is masked by a diffusion component. When it is assumed that at high concentrations of acetate, mediated uptake mechanisms are saturated, a diffusion rate of 0.12 μmoles/g./min. per unit m*M* increase in acetate concentration is calculable from the data in Figure 1, curve A. If the data are corrected for a diffusion component of uptake, a typical adsorption isotherm is obtained (Fig. 1, curve B). However, the data do not rule out conclusively the

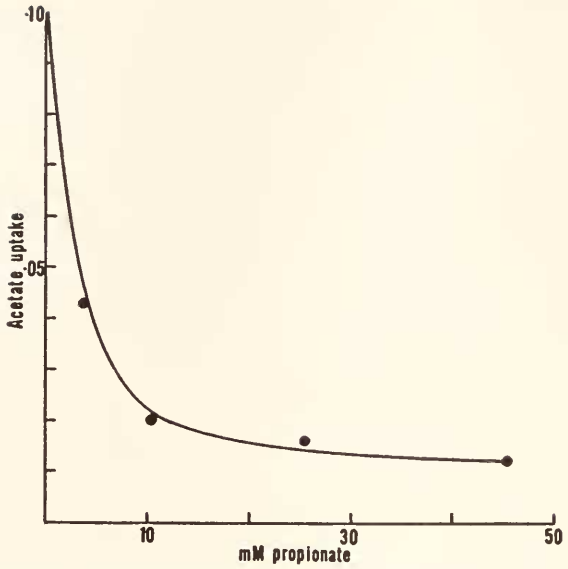

FIGURE 2. The effect of increasing concentrations of propionate on the uptake of acetate (0.1 m*M*). Each point is a mean of 8 determinations.

possibility that a second system for the mediated transport of acetate is involved, since a second system, saturable at very high concentrations, might appear to yield a linear relationship over the concentration range investigated during the present study. The latter probability is diminished, however, by the observation that propionate does not appear to affect the second component of acetate absorption (Fig. 2); the residual absorption of 0.1 m*M* acetate in the presence of 50 m*M* propionate is essentially equal to the diffusion value calculated from the linear portion of the uptake curve shown in Figure 1.

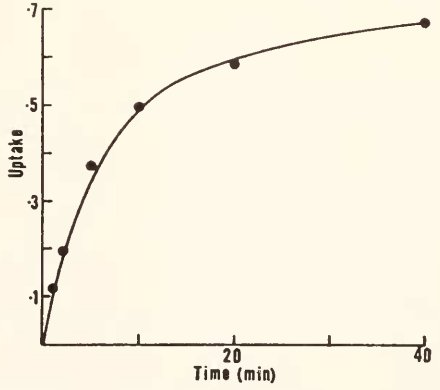

FIGURE 3. Ethanol-extractable ^{41}C, expressed as μmoles ^{14}C-acetate/g, after incubation in 0.1 m*M* ^{14}C-acetate. Each point is a mean of 4 determinations.

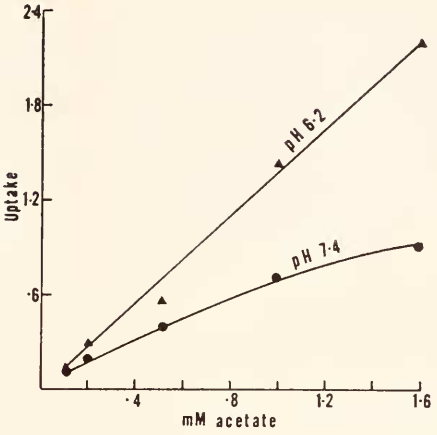

FIGURE 4. The uptake of ^{14}C-acetate at pH 6.2 and 7.4 as a function of acetate concentration. Each point is a mean of 8 determinations.

Analysis of the data on acetate uptake in terms of Michaelis-Menten kinetics allowed an evaluation of K_t (equivalent to Michaelis' constant) and V_{max} (extrapolated maximum velocity). In the concentration range 0.1 to 1.0 m*M* acetate, K_t and V_{max} were 1.13 m*M* and 1.25 μmoles/g./min., respectively, when the data were not corrected for diffusion. After correction, K_t was 0.92 m*M* and V_{max} was 0.91.

The uptake of sodium acetate with respect to time is shown in Figure 3. Following 40 minutes incubation in a substrate concentration of 0.1 m*M* the amount of radioactive material extracted from the worm indicated an internal acetate concentration in the worm water of 0.175 m*M*. Preliminary chromatography indicated that about 60% of the radioactivity extracted was unaltered acetate, but further study would be required to determine more precisely the proportion of the radioactivity representing metabolically unaltered acetate. However, it may be suggested that mediated acetate uptake is a facilitated diffusion rather than active transport.

TABLE I

Effects of pH on acetate uptake and on propionate inhibition of acetate uptake. Acetate concentration 0.1 mM; propionate concentration 4 mM

pH	Uptake (mμmoles/g./min. ± S.E.)		% Inhibition
	Acetate alone	Acetate + propionate	
6.1	161 ± 10.4	134 ± 5.0	17
6.4	147 ± 9.4	102 ± 5.1	31
6.7	139 ± 9.0	64 ± 2.3	54
7.0	135 ± 14.2	53 ± 1.4	61
7.5	129 ± 7.6	39 ± 1.8	70

Influence of pH on acetate absorption

Worms were pre-incubated for 30 min. in KRT at pH 7.4 and then incubated for 1 min. in 0.1 m*M* acetate over a pH range of 6.2–8.0. Radioactivity in incubation solutions was assayed at the end of the experiments in order to determine whether any acetate was lost at low pH; no loss was detectable over the time period of the experiment. Acetate uptake increased as a function of decreasing pH and propionate inhibition of acetate decreased with decreasing pH (Table I). The uptake of acetate at pH 6.2 and 7.4 was studied over a substrate concentration

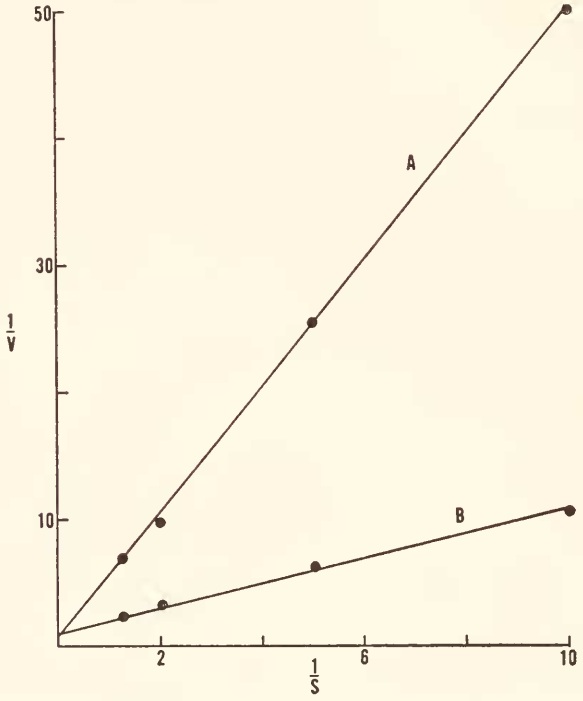

FIGURE 5. The effect of 4 m*M* propionate on the uptake of ^{14}C-acetate in one-minute incubations. A = inhibited slope; B = uninhibited slope; V = uptake, and S = m*M* acetate. Each point is a mean of 12 determinations.

range of 0.1–1.6 m*M* and the results are shown in Figure 4. In contrast to the results obtained at pH 7.4, the uptake of acetate at pH 6.2 was a linear function of acetate concentration.

Effects of other compounds

Effects on acetate uptake of the addition of a variety of compounds were examined. The results of these experiments are shown in Table II. Of the compounds tested, only short chain volatile fatty acids produced a significant effect on acetate uptake.

Acetate uptake at various concentrations in the presence or absence of the individual inhibitory fatty acids of Table II was examined by the Lineweaver-Burke method (1934). A typical double reciprocal plot, that of the effects of propionate on the uptake of acetate, is shown in Figure 5. The approximately common intercept of the slopes of both the inhibited and non-inhibited plots indicates that the inhibition was competitive in nature. Using the K_t and V_{max} values determined experimentally for acetate uptake, the inhibition constants (K_i) for the activity of other fatty acids were determined by application of the equation:

$$\text{Slope} = \frac{K_t}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)$$

TABLE II

Effects of various compounds on acetate uptake in 1 minute. Acetate concentration, 0.1 mM; inhibitor concentration, 4.0 mM

Addition	Uptake (mμmoles/g./min. ± S.E.)	% Inhibition
none	105 ± 6.6	—
2,4-dinitrophenol	102 ± 8.4	0
phlorizin	104 ± 9.2	0
ouabain	107 ± 6.3	0
adenine	101 ± 12.1	0
uracil	102 ± 9.3	0
galactose	96 ± 7.2	0
glucose	107 ± 6.1	0
glycerol	96 ± 4.5	0
alanine	119 ± 11.0	0
phenylalanine	120 ± 10.0	0
arginine	97 ± 6.9	0
glutamic acid	93 ± 7.5	0
leucine	104 ± 6.9	0
lysine	110 ± 10.7	0
betaine	103 ± 8.9	0
sarcosine	121 ± 9.0	0
lactate	91 ± 14.9	0
succinate	124 ± 5.9	0
formate	81 ± 2.3	23
propionate	33 ± 1.8	69
butyrate	53 ± 2.9	50
valerate	63 ± 2.9	40
octanoate	63 ± 1.4	40

Values for the inhibitor constants of the fatty acids studied are: formate 6.9; propionate 1.25; butyrate 3.6; valerate 5.0; and octanoate 5.1.

To determine whether the inhibitions were fully or partially competitive, the uptake of acetate at a concentration of 0.1 m*M* was studied in the presence of inhibitor concentration of 1 to 10 m*M*; the data were then plotted after the method of Dixon (1953). Only formate and acetate were found to be fully competitive inhibitors of acetate uptake.

By an extension of the Michaelis-Menten treatment of enzyme kinetics, Read,

TABLE III

Observed and predicted effects of a mixture of fatty acids on 1-minute acetate uptake. Inhibitors present were sodium propionate (2 mM), sodium butyrate (2 mM), and sodium formate (4 mM)

Acetate concentration (mM)	Uptake mμmoles/g./min. ± S.E.	
	Observed	Predicted
0.1	28 ± 0.6	25
0.2	45 ± 1.7	50
0.4	110 ± 10.2	92
0.6	154 ± 8.6	131

Rothman and Simmons (1963) derived an equation predicting the inhibitory effects of a mixture of amino acids on the uptake of a single amino acid. During the present investigation the effects of a mixture of fatty acids on the uptake of sodium acetate were investigated. Inhibitory effects of a mixture of fatty acids consisting of propionate (2 mM), butyrate (2 mM) and formate (4 mM) on the uptake of acetate at several concentrations are shown in Table III in which observed values are compared to those predicted from the equation:

$$V = \frac{V_{max}}{\frac{K_t}{S} + 1 + \frac{(K_t)\ (I)}{(K_i)\ (S)} + \frac{(K_t)\ (I')}{(K_i')\ (S)} + \frac{(K_t)\ (I'')}{(K_i'')\ (S)}}$$

where V_{max}, K_t, and S are values determined for acetate; K_i, K_i', and K_i'' are independently determined inhibition constants for formate, propionate, and butyrate; and I, I', and I'' are concentrations of the inhibitors.

Uptake of butyrate 1-^{14}C

A limited number of observations were made concerning the relationship between butyrate uptake and concentration between 0.1 and 1.6 mM. Over this concentration range the amount of butyrate absorbed by the tapeworm did not increase

TABLE IV

Relative effects of various fatty acids as inhibitors of acetate and butyrate uptake in 1 minute

Inhibitor	Percentage inhibition	
	^{14}C-acetate	^{14}C-butyrate
Formate	22.9	8.2
Acetate	32.4	16.1
Propionate	69	40.1
Butyrate	50	—
Valerate	40	27.0
Octanoate	40	21.7

linearly with concentration increase and, by methods similar to those outlined above, the K_t and V_{max} values for the butyrate system were 1.5 and 2.5, respectively.

Short chain fatty acids were found to inhibit butyrate uptake and the relative effects of the various inhibitors on both butyrate and acetate uptake are shown in Table IV. The similarity of the relative activity of these several fatty acids as inhibitors of acetate or butyrate uptake suggests similar relative affinities of these inhibitors for the acetate and butyrate transport systems. The uptake of butyrate at several concentrations was determined in the presence and absence of acetate. A Lineweaver-Burke plot of the data so obtained showed the inhibition produced by acetate to be competitive in character and a K_i value of 5.4 for acetate as an inhibitor of butyrate uptake was calculated.

Discussion

It is known that fatty acids enter a number of vertebrate tissues (Fredrickson and Gordon, 1958; Johnston, 1959; Isselbacher, 1965; Quastel, 1965; Hungate, 1966; and others). Quastel (1965) reported that brain tissue accumulated acetate. Transport of acetate, propionate, butyrate, valerate, and hexanoate against a concentration difference has been reported to occur in the small intestine of the rat (Smyth and Taylor, 1958; Barry and Smyth, 1960), although in 60-min. incubations, the concentration ratios reported by the latter workers were always less than 2.0 and the chemical methods used to evaluate the acids were not at all specific (titration after steam distillation).

There is little published information on the kinetics of fatty acid transport. The data of Barry and Smyth (1960) do not permit a conclusion as to whether there is a diffusion component in the absorption of fatty acids by the rat intestine. These workers assumed Michaelis-Menten kinetics and calculated a K_m of 45 m*M*. This may be a spurious value since the data do not show saturation kinetics and may represent a combination of mechanisms for acetate absorption.

The present experiments have shown that a significant proportion of acetate absorption by *Hymenolepis* occurs by a mediated process. At concentrations above 2 m*M*, diffusion appears to be a significant component of acetate uptake. In this dual mode of absorption, the uptake of acetate resembles the uptake of uracil by *Hymenolepis* (MacInnis *et al.*, 1965). Since acetate transport is not affected by a variety of organic compounds, other than fatty acids, it may be concluded that acetate transport in *H. diminuta* occurs through a mechanism with fatty acid specificity. The competitive inhibition of acetate uptake by other short chain fatty acids is further evidence for a specific mediated process and suggests that there is a common transport system for these compounds. Indeed, the similarity in the relative inhibition of acetate or butyrate uptake by the other fatty acids is strong evidence for common transport mechanisms for these two compounds, but more extensive experimentation is required to ascertain whether mediated transport of formate, propionate, valerate, heptanoate, and octanoate occurs only through the acetate-butyrate system.

The effects of pH on acetate uptake suggest that the undissociated form enters more rapidly by diffusion, perhaps because of higher solubility in membrane lipid. The data further suggest that mediated transport mainly involves the dissociated

form of acetate. The effect of pH on the propionate inhibition of acetate is also consistent with the above interpretations.

Ginger and Fairbairn (1966b) remarked that during a 2-hour period the rate of absorption of acetate by *H. diminuta* was linear. Estimation of the concentration of acetate present in Ginger and Fairbairn's experiment (using 2.5 μc. acetate, with specific activity of 2.05 μc./m*M*, in a volume of 10 ml.) indicates a substrate concentration of about 0.121 m*M*. At this concentration, the absorption of acetate during a 2-hour period should be non-linear with time. However, Ginger and Fairbairn's measurements were of the incorporation of acetate into lipid which may not be a measure of absorption. For example, the incorporation of ^{14}C-labeled amino acids into protein in *Hymenolepis* or *Calliobothrium* is linear with time whereas absorption follows first order kinetics (Harris and Read, in preparation; Fisher *et al.*, in preparation). Care must be taken to differentiate absorption from incorporation into other tissue components. It is apparent that in many cases the rate of absorption does not limit the rate of incorporation of a substance. Or, putting it in different terms, the concentrations required to saturate incorporation systems may be quite low when compared with concentrations required to saturate absorption systems. Similarly, the use of the term "uptake" may create confusion. In recent studies on *Mycoplasma*, Rottem and Razin (1967) reported that acetate "uptake" was decreased by butyrate and propionate. These authors speculated that the effect involved inhibition of acetokinase activity, but it is not possible to determine from the data available whether absorption mechanisms or intracellular enzyme reactions are involved. It would be helpful if the term "uptake" were restricted to denote the movement of solute from an extracellular to an intracellular location.

The competition between short chain fatty acids in their absorption by this worm should be considered in terms of (1) the probability that the quality and relative quantities of fatty acids in the small gut are relatively constant and, to some extent, independent of the fatty acids ingested by the host, and (2) the apparent inability of the worm to carry out *de novo* biosynthesis of higher fatty acids (Ginger and Fairbairn, 1966b; Jacobsen and Fairbairn, 1967).

Harrington (1965) showed that the components of *H. diminuta* lipids are quantitatively altered when the worm is reared in hamsters rather than rats. A clue to the basis of such alterations is found in the evidence that a considerable portion of the fatty acids in the lumen of the small intestine are derived from the host (Ginger and Fairbairn, 1966b).

When this is coupled with the finding that *H. diminuta* appears to be quite limited in its ability to synthesize fatty acids, the worms being limited to reactions resulting in chain lengthening (Ginger and Fairbairn, 1966; Jacobsen and Fairbairn, 1967), specific characteristics of fatty acids of host origin should be reflected in the lipid composition of the worm. Tentative acceptance of this interpretation leads to the conclusion that the transport of fatty acids into the tissues of the worm may be of considerable importance in determining the lipid composition and, in this context, the competitions between fatty acids observed in the present study assume more meaning.

Acetate has been reported to be an end-product of carbohydrate metabolism in *H. diminuta* and to be excreted into the external medium by the worm (Fair-

bairn *et al.*, 1961). It might be argued that acetate absorption is of no physiological significance since the worm produces the compound in considerable quantity. However, it has been shown that labeled acetate in the external medium enters the tissues and is incorporated into tissue lipids (Ginger and Fairbairn, 1966b; Jacobsen and Fairbairn, 1967). The latter authors remarked that the incorporation of ^{14}C-acetate occurred at a much lower rate than the incorporation of higher fatty acids. This might be attributable to dilution of the labeled acetate by the unlabeled acetate originating from carbohydrate metabolism. On the other hand, Ginger and Fairbairn (1966b) observed that only small amounts of ^{14}C previously incorporated into glycogen appeared in lipid during a prolonged incubation in which most of the glycogen was metabolized. This does not appear to be completely consistent with the view that significant portions of the acetate produced endogenously are incorporated into lipid. Further investigation of the relative significance of acetate from metabolic and external sources would be desirable. The present authors can offer no explanation for Daugherty's (1957) report that significant amounts of acetate do not enter the tissues of *H. diminuta* in 15-min. incubations at 37° C.

It is tacitly recognized that the absorption of fatty acids by the worm living in a host may involve important modifications not duplicated in the present experiments. There is evidence that, in the digestive tract, fatty acids, monoglycerides, and conjugated bile salts are present as mixed micelles (Hofmann and Borgström, 1962). This may be of great significance in the absorption of higher fatty acids which are relatively insoluble in water, even at soaps. Thus, while bile may serve as a source of fatty acids (Baxter, 1966), it also contributes bile salts which may be of significance in fatty acid absorption. An investigation of the effects of bile salts on absorption of fatty acids by *Hymenolepis* will be the subject of a subsequent portion of this study. The effects of pH on fatty acid uptake suggest that the antero-posterior pH gradient in the small intestine of the host may be significant in determining the rate of fatty acid uptake and the relative roles of mediated transport and diffusion.

Of a number tested, the only compounds producing competitive or partially competitive inhibition of acetate or butyrate uptake were other volatile fatty acids. This supports the view that the mediated transport of these fatty acids occurs through mechanisms showing some specificity. As a matter of fact, four types of mediated transport showing chemical group specificity are now known in *Hymenolepis diminuta*. Group A includes mechanisms for the mediated transport of amino acids (Read, Rothman and Simmons, 1963); Group B includes mechanisms for the mediated transport of monosaccharide sugars (Phifer, 1960a, b; Read, 1961); Group C includes mechanisms for the transport of purine and pyrimidine bases (MacInnis, Fisher and Read, 1965); and Group D, identified in the present work, includes mechanisms for transport of short chain fatty acids. At higher concentrations, considerable quantities of the compounds of Groups C and D enter the worm tissues by diffusion. Preliminary observations suggest that higher fatty acids, such as palmitic, may be absorbed by *H. diminuta* through mechanisms which are independent of those involved in the absorption of the short chain fatty acids. Transport of higher fatty acids is being examined in some detail.

The technical assistance of Miss Linda J. Rogers is gratefully acknowledged.

Summary

1. At low substrate concentrations (below approximately 2 m*M*) *Hymenolepis diminuta* absorbs acetate by a mediated process; at high substrate concentrations the main mode of entry is diffusion.

2. Hydrogen ion concentration affects acetate uptake. At low pH the rate is increased and there is a larger diffusion component. Inhibition of acetate uptake by propionate is depressed at low pH.

3. Acetate uptake is inhibited by other short chain fatty acids but is unaffected by a variety of other compounds. The kinetics of the inhibitions were examined and inhibitions were found to be partially competitive in the case of propionate, butyrate, valerate and octanoate and fully competitive in the case of formate. Butyrate uptake is also inhibited by these compounds.

4. The data are discussed in terms of transport of fatty acids, lipid metabolism in cestodes and possible significance in the ecology of the organism.

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